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Jun 4, 2002

TITLE: Shc-binding protein

The predicted amino acid sequence encoded by mPAL DNA contains 23 tyrosine residues, several of which are embedded in consensus binding motifs for SH2 domains. In addition, two highly acidic regions are encoded by the mPAL DNA. Comparison of both the nucleotide and protein sequences of mPAL with the GenBank databases revealed no significant homology between mPAL and any previously identified proteins. We have identified several related expressed sequence tags (ESTs) represent human and rat homologues of mPAL. In addition several short murine and human ESTs with approximately 50% sequence similarity to regions of mPAL were identified, suggesting that additional mPAL related genes exist.

The full length PAL polypeptide or fragment thereof can be prepared using well known recombinant DNA technology methods such as those set forth in Sambrook et al. Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989) and/or Ausubel et al., eds, Current Protocols in Molecular Biology, Green Publishers Inc. and Wiley and Sons, NY (1994). A gene or cDNA encoding the PAL protein or fragment thereof may be obtained for example by screening a genomic or cDNA library, or by PCR amplification. Alternatively, a gene encoding the PAL polypeptide or fragment may be prepared by chemical synthesis using methods well known to the skilled artisan such as those described by Engels et al., Angew. Chem. Intl. Ed., 28:716-734 (1989). These methods include, inter alia, the phosphotriester, phosphoramidite, and H-phosphorate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding the PAL polypeptide will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full length PAL polypeptide. Usually, the DNA fragment encoding the amino terminus of the polypeptide will have an ATG, that encodes a methionine residue. This methionine may or may not be present on the mature form of the PAL polypeptide, depending on whether the polypeptide produced in the host cell is secreted from that cell.

In some cases, it may be desirable to prepare nucleic acid and/or amino acid variants or analogs of naturally occurring PAL. Nucleic acid variants or analogs (wherein one or more nucleotides and/or amino acids are designed to differ from the wild-type or naturally occurring PAL) may be produced using site directed mutagenesis or PCR amplification where the primer(s) have the desired point mutations (see Sambrook et al., supra, and Ausubel et al., supra, for descriptions of mutagenesis techniques). Chemical synthesis using methods described by Engels et al., supra, may also be used to prepare such variants. Other methods known to the skilled artisan may be used as well. For example, in Wayne et al., EMBO J 2:1827-1829 (1983), the authors teach a method for deletion mutagenesis, that was used to generate mutants of the TyrTS gene. Huang et al., Cell 48:129-136 (1987), analyzed

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Other Reference Publication (78):

Other Reference Publication (82):

Adams, M.D. et al., "Rapid cDNA sequencing (expressed sequence tags) from a directionally cloned human infant brain cDNA library," Nature Genetics, 4:373-380 (Aug., 1993).